J Forensic Sci, July 2009, Vol. 54, No. 4
doi: 10.1111/j.1556-4029.2009.01045.x
Available online at: www.blackwell-synergy.com

Amy Z. Mundorff,[1,‡] *M.A.; Eric J. Bartelink,*[2] *Ph.D.; and Elaine Mar-Cash,*[1,§] *M.S.*

DNA Preservation in Skeletal Elements from the World Trade Center Disaster: Recommendations for Mass Fatality Management*,†

ABSTRACT: The World Trade Center (WTC) victim identification effort highlights taphonomic influences on the degradation of DNA from victims of mass fatality incidents. This study uses a subset of the WTC-Human Remains Database to evaluate differential preservation of DNA by skeletal element. Recovery location, sex, and victim type (civilian, firefighter, or plane passenger) do not appear to influence DNA preservation. Results indicate that more intact elements, as well as elements encased in soft tissue, produced slightly higher identification rates than more fragmented remains. DNA identification rates by element type conform to previous findings, with higher rates generally found in denser, weight-bearing bones. However, smaller bones including patellae, metatarsals, and foot phalanges yielded rates comparable to both femora and tibiae. These elements can be easily sampled with a disposable scalpel, and thus reduce potential DNA contamination. These findings have implications for DNA sampling guidelines in future mass fatality incidents.

KEYWORDS: forensic science, forensic anthropology, forensic taphonomy, DNA identification, mass fatality incident, victim identification, terrorism, World Trade Center, 9/11

On the morning of September 11, 2001, two commercial airliners (American Airlines Flight 11, United Airlines Flight 175) were flown into the 110-story North and South Towers of the World Trade Center (WTC) in New York City as part of a terrorist attack on the United States. The impact of the airliners and subsequent collapse of the seven buildings that comprise the WTC complex resulted in the deaths of 2749 victims and involved an initial recovery effort that lasted over 9 months (1). During this period, the victims' remains were differentially exposed to a variety of taphonomic factors. These include UV radiation, humidity, moisture, heat, fire, and mold—all of which contributed to the advanced state of decomposition of the remains and to the degradation of DNA. With nearly 20,000 individual sets of human remains recovered, the WTC disaster represents one of the most comprehensive victim identification efforts undertaken to date (2). The goals of this study are to: (i) examine DNA typing success rates to provide a better understanding of differential degradation of genetic material between skeletal elements; and (ii) to provide sampling protocol guidelines for future mass fatality incidents.

DNA Sampling Protocols

Since the 1990s, DNA has played an increasingly important role in the identification of victims of mass fatality incidents (3–13). Recent events such as the September 11, 2001 terrorist attacks, the 2004 Boxing Day tsunami, and the 2005 Hurricane Katrina disaster demonstrate that DNA identification, where resources are available, has supplemented more traditional forms of identification such as dental and fingerprint comparisons. However, DNA preservation in human remains may be influenced by a complex interaction of taphonomic processes, including exposure of remains to moisture, humidity, UV radiation, fire, microbes, flora, fauna, and soil (14).

Previous studies have found that genetic material degrades more rapidly in soft tissue than in bone, due to the more resilient structure of bone acting as a physical barrier against taphonomic influences (15–18). Bone density is also an important factor influencing bone preservation (19). Hence, DNA is usually less degraded in the denser portions of the skeleton, especially weight bearing elements such as the femur and tibia (20–22). However, few studies have systematically addressed differences in DNA typing success rates between skeletal elements. Published data suggest that DNA is more well-preserved in clavicles than in rib bone (20), in long bones than in skull or rib bone (22), in weight-bearing long bones than in nonweight-bearing elements (23,24), and in compact bone than in cancellous bone (25). Milós et al. (24) reported DNA typing success rates for 25,361 bone and tooth specimens recovered from mass grave victims in the former Yugoslavia, buried for an interval of 4–11 years. Their results indicate that DNA is best preserved in femora and teeth, followed by tibiae, fibulae, humeri, crania, radii, and ulnae. These findings are in general agreement with the taphonomic literature that shows a robust relationship between bone density and skeletal preservation (26). It is clear that DNA typing success rates vary between skeletal elements, although the reasons for this are not well understood. Leney (27) has argued that areas under high mechanical load, such as the mandible and weight-bearing postcranial elements, contain thicker cortical bone that may act as a protective barrier against DNA degradation. For

[1]Office of Chief Medical Examiner, New York City, NY.
[2]Department of Anthropology, California State University, Chico, 400 West First Street, Chico, CA 95929-0400.

*An earlier version of this paper was presented at the 59th Annual Meeting of the American Academy of Forensic Sciences, San Antonio, TX, February 19–24, 2007.

†Funded by a Trudeau Foundation Scholarship and a Special Graduate Entrance Scholarship from Simon Fraser University.

‡Present address: Amy Z. Mundorff, Department of Archaeology, Simon Fraser University, 8888 University Drive, Burnaby, BC V5A 1S6, Canada.
§Present address: Elaine Mar-Cash, Holden Dr., Ann Arbor, MI.

Received 6 Feb. 2008; and in revised form 2 Aug. 2008; accepted 3 Aug. 2008.

teeth, however, it is the tooth enamel that protects DNA from degradation and contamination (24).

Despite a growing trend towards DNA-based identification, there are no detailed guidelines for sampling biological remains for DNA analysis in the mass fatality literature. DNA sampling protocols used in recent mass fatality incidents vary and are often tailored to the unique circumstances of the disaster. For example, muscle tissue and rib bone was sampled from the 1995 Branch Davidian victims in Waco, Texas (28); femora were sampled from victims of the 2002 Bali nightclub bombings (29); ribs and teeth were initially sampled from victims of the 2004 tsunami in Phuket, Thailand (30); and the anterior tibial midshaft was sampled for the 2005 victims of Hurricane Katrina (D. Boyer, personal communication, 2006).

To address disparities in sampling strategies, agencies have begun issuing guidelines and recommendations for DNA sampling. The National Institute of Justice (NIJ) recently developed "Mass Fatality Incidents: A Guide for Forensic Human Identification" (31), which addresses DNA sampling methods. This guide provides general sampling guidelines, stating that, "the sampler obtains one of the following, listed in order of preference": deep skeletal muscle, cortical bone, canine tooth, or other portion of soft or hard tissue (31). In 2007, the DNA Commission of the International Society for Forensic Genetics (ISFG) published, "Recommendations Regarding the Role of Forensic Genetics for Disaster Victim Identification (DVI)." Of the twelve recommendations, number three addresses postmortem sampling, again confirming a preference for dense cortical bone, particularly from weight-bearing leg bones (21). However, neither guideline details specifically which bone to sample in order to maximize the success of a DNA profile. These existing guidelines offer only very broad recommendations, and do not detail which bones are most likely to consistently produce DNA profiles under adverse taphonomic conditions.

Goals of Study

The goal of this study is to supplement and expand upon previous DNA sampling recommendations (21,24,28,31). We specifically address variation in DNA typing success rates between skeletal elements and provide recommendations for DNA sampling protocols for future victim identification efforts associated with mass fatality incidents. In this paper, a subset of the WTC-Human Remains Database (WTC-HRD) is used to examine differences in the DNA identification rates between skeletal elements. We also consider how sex, victim type (WTC civilian, plane passenger, firefighter), and recovery location (WTC site, Staten Island landfill) affect DNA typing success rates. In many ways, the WTC disaster represents a "worst case scenario" in that remains were exposed to a wide variety of taphonomic factors over a long postmortem interval. However, many victim identification efforts are confronted with similar challenges and this study should provide a broad basis for comparison.

Materials and Methods

This study examines two data subsets from the WTC-HRD, the complete database consisting of 19,970 sets of human remains (as of September 2005). These include the Resampled Dataset (RD) and the Entire Sample Dataset (ESD). The RD (n = 537) consists of remains that initially failed to produce sufficient DNA profiles but were later resampled and retested. In many instances, soft tissue (e.g., muscle, skin) was initially sampled. In these resampled cases, a specific skeletal element was selected for retesting. The criteria for selection included macroscopic preservation of the external surface (e.g., no evidence of burning, severe fragmentation, weathering, embedded soil or concrete dust), a preference for dense cortical bone over cancellous bone, long bones over axial elements, and bone still protected by soft tissue over completely skeletonized elements. However, cases that consisted only of poorly preserved bone were still sampled.

The ESD (n = 3052) consists of smaller bone specimens that fit within a 50 ml sample vial. These samples are predominantly small bone fragments of different elements found in isolation during the recovery effort. ESD samples were submitted in their entirety for DNA testing, and in some cases, testing consumed the entire specimen. The RD and ESD were chosen for analysis from the complete WTC-HRD because the specific element tested for DNA had been recorded in detail at the time of sampling for each case instead of the sample generically labeled as "muscle" or "bone." This permitted the retrospective categorization of successful DNA typing rates by skeletal element.

The RD and ESD were initially examined separately to address potential differences in each dataset. In a second analysis, both datasets were combined to create the Complete Elements Dataset (CED). Cases that consisted of soft tissue, hair, teeth, indeterminate bone fragments, and calcined remains were excluded from the analysis. For ease of comparison, metacarpals are grouped together, metatarsals are grouped together, and proximal, intermediate, and distal phalanges are grouped together, the latter group categorized as either "hand" or "foot" phalanx. Similarly, all carpal elements and all tarsal elements are combined for the hand and foot, respectively. Finally, all vertebral segments are combined. The individual element categories are also collapsed into the following groups for further analysis by "body part group":

1. Head: skull, maxilla, mandible (excluding teeth)
2. Trunk: clavicle, scapula, rib, sternum, vertebra, sacrum, innominate
3. Upper limb: humerus, radius, ulna, carpal, metacarpal, hand phalanx
4. Lower limb: femur, patella, tibia, fibula, tarsal, metatarsal, foot phalanx

A number of variables can influence degradation of DNA in mass fatality incidents, such as intrinsic properties of skeletal elements (e.g., cortical thickness), age and sex differences in bone density, location of the victim within the disaster site, and the context of recovery. In this study, we consider the success of DNA typing rates by skeletal element, sex of victim, recovery location, and victim type (Table 1). The month of recovery is not examined in this study since DNA testing did not always occur immediately following the discovery of the remains from either the WTC site in Manhattan or the Fresh Kills Landfill on Staten Island.

DNA Identification Criteria

In the complete WTC-HRD, several cases were identified by multiple modalities (e.g., both DNA and dental identifications); however, only DNA-based identifications are considered in this

TABLE 1—*Variables examined in WTC-HRD.*

Variable	Variable Description
Element	Skeletal element sampled
Sex of victim	Male, Female
Victim type	WTC Civilian, Firefighter, Plane victim
Recovery location	WTC site (Manhattan), Fresh Kills Landfill (Staten Island)
DNA identification	Yes, No

paper. For this study, all identified samples are considered successfully typed using DNA testing; thus, we use the term *identified* synonymously with *successful DNA typing*. As of September 2005, WTC samples were subjected to short-tandem repeat (STR) DNA analysis using the standard loci of the Combined DNA Index System (CODIS) (32,33) and the additional two Penta loci from the PowerPlex® 16 commercial kit (34), mitochondrial DNA sequencing of the HVI and HVII regions (35), and single nucleotide polymorphisms (SNP) analysis (a contract laboratory customized a unique 70 SNP panel for the WTC samples, B. Giles, O. Cellmark, personal communication, 2003). The population frequencies for all available STR and SNP profiles and available mitotypes were calculated and multiplied together to obtain a final frequency, which was compared to a set minimum threshold requirement for identification. This threshold was determined to achieve a standard of less than 1 chance in 1,000,000 that a misidentification would result from a fortuitous match in a population estimated at the time to be 5000 victims, regardless of the number of markers that produced genetic data (36). As the number of victims was lowered from 5000 to 2749, this value was also lowered to 2×10^9, 2×10^8, and 4×10^9, for males, females, and profiles with no amelogenin results, respectively. Samples were subsequently identified by direct comparison to a victim exemplar type and/or through kinship analysis using family reference types.

DNA testing performed on bones was undertaken by a subcontracted laboratory and the Department of Forensic Biology at the Office of Chief Medical Examiner, New York City.

Statistical Analyses

Chi-square tests are used to evaluate the relationship between DNA identification rates by recovery location and between skeletal elements in the RD and ESD. In many cases, multiple bone fragments were linked to a single individual through DNA. Thus, comparisons of DNA identification rates focus on variation between different skeletal elements instead of individual victims. For the variables *sex of victim* and *victim type* (which comprise only positively identified victims for which sex and victim type are known), frequencies are compared to determine whether DNA-based identification of bone fragments in the RD and ESD occurred in the same proportion as in the complete WTC-HRD and in the total missing victim population. Statistical analyses were computed using SPSS Version 15.0 (SPSS Inc., Chicago, IL) and significance was set at $\alpha = 0.05$.

Results

Comparison of Resampled and Entire Sample Datasets

The RD comprises cases from the complete WTC-HRD that had been resampled for DNA analysis. To facilitate comparison, elements with small sample sizes ($n < 15$) were removed from the dataset for all statistical analyses (e.g., carpals, tarsals, hand and foot phalanges, ulnae, scapulae, maxillae, sterna, and sacra), leaving 537 (of 641) cases for analysis (Table 2). The ESD comprises cases from the complete WTC-HRD recorded as "Entire Sample." Again, elements with small sample sizes ($n < 15$) were removed (e.g., carpals, patellae, and sterna), leaving 3052 cases (of 4664) for analysis (Table 3). Costal cartilage is not included in statistical comparisons; however, it is worth noting that 72% of cartilage samples from the ESD were identified using DNA ($n = 43$).

Although the RD and ESD differ in sample size ($n = 537$ vs. $n = 3052$, respectively), DNA identification patterns are surprisingly similar. For example, successful DNA typing rates by sex and victim type (WTC civilian, plane passenger, and firefighter) are nearly identical within the different variables (Tables 4 and 5). However, there are slight differences between datasets. Most noticeably, nearly all skeletal elements show higher DNA identification rates in the RD than in the ESD (e.g., humerus, radius, rib, innominate, tibia, fibula, metatarsal, and metacarpal; Tables 2 and 3). Overall, 71% of samples in the RD were

TABLE 2—*Identification statistics for RD.*

Element	*n*	Number Identified	Percent Identified (%)
Metatarsal	42	36	86
Patella	78	63	81
Tibia	43	33	77
Metacarpal	21	16	76
Innominate	19	14	74
Femur	66	47	71
Rib	45	32	71
Mandible	26	18	69
Radius	25	17	68
Fibula	38	25	66
Humerus	60	39	65
Vertebra	18	11	61
Clavicle	19	11	58
Skull	37	18	49
Total	537	380	71

TABLE 3—*Identification statistics for ESD.*

Element	*n*	Number Identified	Percent Identified (%)
Foot phalanx	24	19	79
Femur	77	55	71
Metatarsal	215	148	69
Tibia	82	55	67
Rib	1256	806	64
Vertebra	54	33	61
Mandible	20	12	60
Fibula	121	71	59
Sacrum	24	14	58
Innominate	43	25	58
Radius	95	55	58
Hand phalanx	81	46	57
Humerus	50	28	56
Tarsal	31	17	55
Ulna	74	40	54
Clavicle	78	41	53
Scapula	80	42	53
Skull	457	214	47
Metacarpal	190	77	41
Total	3052	1798	59

TABLE 4—*Male–female identification rates by dataset.*

	Sex	
Datasets	Male (%)	Female (%)
RD	80	20
ESD	82	18
CED	83	17
WTC-HRD	84	16
Total missing victims	77	23
Identified victims	80	20

TABLE 5—*Victim location identification rates by dataset.*

Datasets	Victim Type		
	Firefighter (%)	Plane (%)	Civilian (%)
ESD	12	4	84
RD	14	3	83
CED	12	4	84
WTC-HRD	13	5	82
Total missing victims	13	5	82

identified through DNA compared to 59% in the ESD. However, overall identification rates are not directly comparable because the ESD includes elements not represented in the RD. These results suggest that there are likely different underlying taphonomic factors influencing DNA degradation in each dataset. For example, samples in the RD were carefully selected (during the resampling process) based on macroscopic preservation and were often protected by skin or soft tissue. In contrast, samples in the ESD were mainly represented by smaller, isolated bone fragments devoid of soft tissue.

Chi-square tests were used to compare DNA identification rates between the same skeletal elements in the two datasets (Table 6). Although all elements in the RD have higher identification rates compared with the ESD, these differences are only statistically significant for the metacarpal ($\chi^2 = 9.758$, $p = 0.002$, $\phi = 0.215$) and metatarsal ($\chi^2 = 4.921$, $p = 0.027$, $\phi = 0.138$). Phi coefficients for these comparisons indicate a weak relationship (<0.3), suggesting that the disparity in sample size between the two datasets may account for some of the difference in the DNA identification rates. However, some disparity between the RD and the ESD was expected, since the RD samples were selected specifically based on macroscopic preservation and the particular elements available for resampling in each case. These results suggest that elements encased in soft tissue are more likely to produce successful DNA typing than those that consist of isolated bone fragments.

When examined by "body part group," a similar discrepancy emerges (Table 7). Chi-square tests computed for each body part group (head, trunk, upper limb, and lower limb) indicate significant differences for the upper limb ($\chi^2 = 17.035$, $p = 0.001$, $\phi = 0.167$) and lower limb ($\chi^2 = 7.371$, $p = 0.007$, $\phi = 0.094$). The weak Phi coefficients values (<0.3) again suggest that these differences may be influenced by the disparity in sample size between the two datasets and by differences in the DNA identification rates for the metacarpal and metatarsal. Although differences exist between the RD and the ESD, the two datasets show similar DNA typing rates. Thus, the RD and ESD are combined to create the CED, which is treated as more representative of the total WTC-HRD.

Complete Elements Dataset

The CED comprises the combined RD and ESD ($n = 3631$), representing *c.* 18% of the entire WTC-HRD sample ($n = 19{,}970$). Elements with small sample sizes ($n < 15$) were removed from the dataset (e.g., carpals, sterna, and maxillae). Comparison of DNA identification rates between the CED and WTC-HRD are remarkably similar. For example, 56% of cases are identified in the CED compared with 57% in the total WTC-HRD. A total of 2749 victims are reported missing, 2122 (77%) of which are male and 627 (23%) of which are female (Table 4; Fig. 1). Of the 1598 victims that have been positively identified, 1272 (80%) are male and 326 (20%) are female. This closely approximates the demographics of

TABLE 6—*Comparison of resampled and entire sample dataset by element type.*

Element	χ^2-Value	*p*-value*	Phi	RD versus ESD (number identified/ number tested)	Percent Identified in RD versus ESD
Clavicle	0.175	0.676		11/19 versus 41/78	57.9 versus 52.6
Femur	0.001	0.977		47/66 versus 55/77	71.2 versus 71.4
Fibula	0.611	0.434		25/38 versus 71/121	65.8 versus 58.7
Humerus	0.928	0.335		39/60 versus 28/50	65.0 versus 56.0
Mandible	0.425	0.515		18/26 versus 12/20	69.2 versus 60.0
Metacarpal	9.758	**0.002**	0.215	16/21 versus 77/190	76.2 versus 40.5
Metatarsal	4.921	**0.027**	0.138	36/42 versus 148/215	85.7 versus 68.8
Innominate	1.365	0.243		14/19 versus 25/43	73.7 versus 58.1
Radius	0.842	0.359		17/25 versus 55/95	68.0 versus 57.9
Rib	0.913	0.339		32/45 versus 806/1256	71.1 versus 64.2
Skull	0.046	0.831		18/37 versus 214/457	48.6 versus 46.8
Tibia	1.266	0.261		33/43 versus 55/82	76.7 versus 67.1
Vertebra	0.000	1.00		11/18 versus 33/54	61.1 versus 61.1

*Bold-face *p*-values are significant at ≤ 0.05.

TABLE 7—*Comparison of RD and ESD by body part group.*

Body Part Group	χ^2-Value	*p*-value*	Phi	RD versus ESD (number identified/ number tested)	Percent Identified in RD versus ESD
Head	2.124	0.145		36/63 versus 224/477	57% versus 47%
Trunk	0.993	0.319		78/116 versus 967/1535	67% versus 63%
Upper limb	17.035	**0.001**	0.167	86/121 versus 245/490	71% versus 50%
Lower limb	7.371	**0.007**	0.094	208/274 versus 366/555	76% versus 66%

*Bold-face *p*-values are significant at ≤0.05.

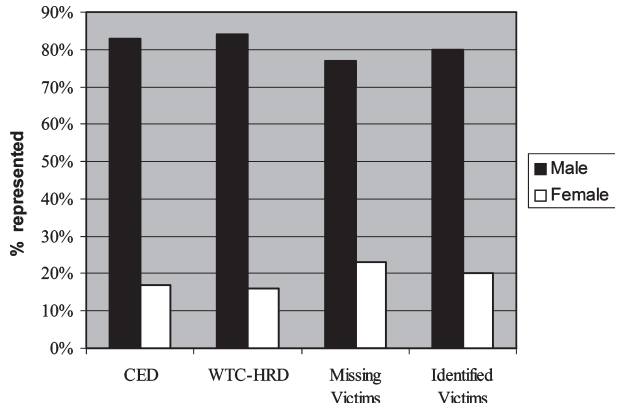

FIG. 1—*Bar chart comparing the sex ratio of identified remains in the Complete Elements Dataset (CED), the WTC-Human Remains Database, the WTC Missing Victims List, and the WTC Identified Victims List.*

the total WTC victim population. Examination of the 10,927 positively identified sets of remains from the WTC-HRD indicates that 84% are male and 16% are female. Similarly, 83% of the identified remains in the CED are males and 17% are females (Table 4; Fig. 1). Identification rates compared by victim type are also nearly identical between the CED and WTC-HRD, both of which mimic the WTC missing victim list (Table 5; Fig. 2). In other words, identification rates in the CED closely approximate the identification rates in the WTC-HRD as well as the total WTC victim population. This suggests that the CED is a representative sample of the WTC victim population.

Results of Individual Skeletal Elements from the CED

Both intrinsic and extrinsic factors influence the differential preservation of DNA in skeletal remains. As discussed previously, human remains of identified males and females gave successful DNA typing rates in nearly the same proportions that each sex is represented in the total missing victim population (Table 4; Fig. 1). Victim type also did not seem to have an influence on DNA typing rates, since WTC civilians, plane passengers, and firefighters were identified in nearly the same proportions as represented in the total missing victim population (Table 5; Fig. 2). However, recovery location showed slight differences in DNA typing results. At the WTC site, where the majority of remains were recovered, 61.6% of skeletal elements were identified. This is slightly higher than the remains recovered from the Staten Island landfill, where 56.9% of skeletal elements were identified. Although a significant difference ($\chi^2 = 4.622$, $p = 0.032$; $\phi = 0.036$), Phi coefficients indicate a weak strength of association (< 0.3) indicating that the large sample sizes are only detecting a small difference in DNA typing rates.

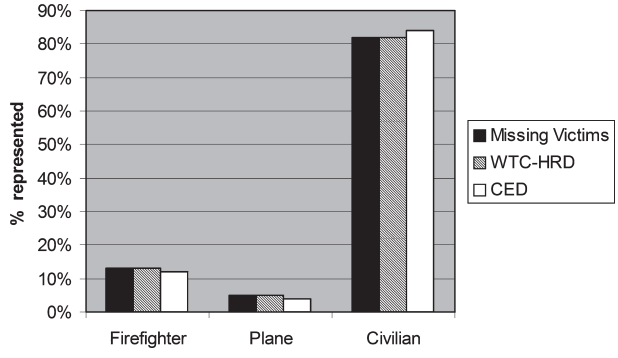

FIG. 2—*Bar chart comparing the percentage of identified remains by victim type in the Missing Victims List, the WTC-Human Remains Database, and CED.*

These results suggest that sex, victim type, and recovery location are not significant variables influencing DNA identification rates for the WTC disaster. Differences among skeletal elements are next examined in the CED as a whole. Table 8 provides a complete list of DNA identifications for the 3631 elements reported in descending order. The foot phalanx and patella show the highest DNA identification rate at 80%, followed by the metatarsal, femur, and tibia, which fall within the 70th percentile. Elements that fall within the 60th percentile include the mandible, rib, innominate, vertebra, humerus, ulna, fibula, and radius. Finally, the sacrum, hand phalanx, scapula, clavicle, tarsal, skull, and metacarpal show the lowest rates, falling within the 40th to 50th percentiles.

When compared by body part group, DNA identification rates are as follows: lower limb = 69%; trunk = 63%; upper limb = 54%; and head = 49%. These rates are significantly different between the lower limb and the trunk ($\chi^2 = 10.038$, $p = 0.002$), between the trunk and the upper limb ($\chi^2 = 13.797$, $p = 0.001$), and between the upper limb and the head ($\chi^2 = 3.886$, $p = 0.049$; Table 9). These results indicate that the lower limb shows a slightly higher DNA identification rate compared with other body part groups, although the strength of this relationship is weak ($\phi < 0.3$).

Discussion

One of the goals of this study was to examine differences in DNA typing success rates between skeletal elements to provide a basis for establishing general sampling guidelines for future mass fatality incidents. The most recent guidelines issued by the NIJ and the DNA Commission of the ISFG regarding DNA sampling methods from mass fatality incidents do not address which skeletal elements are most likely to result in successful DNA testing (21,31). Guidelines must account for the degradation of genetic material resulting from taphonomic factors, and provide recommendations for sampling fleshed versus skeletonized remains. This highlights the need for additional data on DNA results for different skeletal

TABLE 8—*Identification statistics for CED.*

Element	*n*	Number Identified	Percent Identified (%)
Foot phalanx	25	20	80
Patella	83	66	80
Metatarsal	257	184	72
Femur	143	102	71
Tibia	125	88	70
Mandible	46	30	65
Rib	1301	838	64
Innominate	62	39	63
Vertebra	72	44	61
Humerus	110	67	61
Ulna	87	53	61
Fibula	159	96	60
Radius	120	72	60
Sacrum	27	16	59
Hand phalanx	83	47	57
Scapula	92	50	54
Clavicle	97	52	54
Tarsal	37	19	51
Skull	494	232	47
Metacarpal	211	93	44
Total	3631	2208	61

TABLE 9—*Comparison of complete elements dataset by body part group.*

Body Part Group	χ^2-Value	*p*-value*	Cramer's V	RD versus ESD (number identified/ number tested)	Percent Identified in RD versus ESD
Lower limb versus trunk	10.038	**0.002**	0.064	829 versus 1651	69% versus 63%
Trunk versus upper limb	13.797	**0.001**	0.078	1651 versus 611	63% versus 54%
Upper limb versus head	3.886	**0.049**	0.058	611 versus 540	54% versus 49%

*Bold-face *p*-values are significant at ≤0.05.

elements recovered from a variety of mass fatality contexts. For example, a DNA sampling protocol for intact bodies would differ significantly from a protocol for heavily fragmented and burned remains from an airline disaster. The results of this study may be useful in establishing general guidelines for DNA sampling protocols that can be adapted to a variety of mass fatality scenarios. However, these guidelines may not be appropriate for DNA sampling of remains recovered from mass graves (24). This study focuses on DNA results from recently deceased victims of mass disasters, and may not encompass the range of taphonomic factors that affect buried remains.

The DNA results from WTC victims indicate that lower limb elements (fibula excluded) are more likely to generate successful DNA results than elements of the upper limb and axial skeleton. This is likely due to the weight-bearing properties of the lower limb, which produce denser cortical bone that protects against DNA degradation. The high DNA identification rate for the patella in particular may be due to increased density resulting from high functional stress demands of locomotion (27). Skeletal elements that are encased in soft tissue also appear to be better protected and are likely to produce more successful DNA typing results than isolated bone fragments. This may account for the differences in identification rates of the metacarpal between the RD and the ESD (RD = 76.2% vs. ESD = 44.1%), since samples from the RD were more likely to have been sampled from within soft tissue.

In general, weight-bearing lower limb elements produced successful DNA profiles at higher rates than elements from the trunk, the upper limb, and the head. Examination of the elements from all datasets indicates that the patella, metatarsal, and foot phalanx are among the top identified elements. Most notably, the metatarsal and patella are the only elements from the Resampled Dataset that resulted in DNA profiles with a success rate greater than 80% (86% and 80.8%, respectively). The tibia, femur, and rib followed closely (77%, 71%, and 71%, respectively).

Although the relatively similar success rates between some elements (80.8% patella vs. 77% tibia) would seem to belie any strong preference for sampling, practical management considerations should be aimed at reducing potential DNA contamination. Because patellae, metatarsals, and foot phalanges had successful DNA typing rates comparable to long bones, these elements may be ideal for sampling due to the ease with which they can be removed and their relative imperviousness to contamination. Femora, tibiae, and ribs are among the more common elements sampled in previous mass fatality incidents (28–30,37). However, sampling these elements, especially from relatively intact bodies, may require the use of an electric bone saw or hacksaw. Many mass fatality incidents occur in areas where electricity is not readily available, which may limit mortuary operations to facilities with electricity or access to generators. Additionally, the use of any type of bone saw is labor intensive for sampling, and the blade must be changed between samples or thoroughly cleaned with a bleach-water solution to prevent DNA contamination.

The high DNA identification rates for the foot phalanx, patella, and metatarsal suggest that these may be ideal elements for DNA sampling of relatively intact bodies. Additionally, preferential sampling of these elements when possible takes into account a number of practical management considerations. First, these bones can be easily removed as intact elements, instead of sampling from an open section of a long bone, which may introduce DNA contamination to the sample. Further, these elements can be removed using a scalpel, eliminating the need for a bone saw (and electricity) and reducing the potential for cross-contamination between sampling episodes. Finally, scalpels are inexpensive, disposable, readily available, and less labor intensive for sampling. Previous research on the WTC victim identification effort shows that DNA contamination can be a serious problem in mass fatality incidents (3,13). Thus, all possible measures should be taken to minimize the potential for sample contamination.

Conclusions

There are a number of limitations to the current study. The datasets used may not be entirely representative of the total WTC victim population, and also may not reflect the full range of taphonomic conditions that affected DNA preservation. Additionally, this study was not able to address the influence of time-since-death because remains were recovered and DNA tested at different times. Finally, it should be noted that the identification process relied on successful DNA typing of not only the remains, but also the comparison exemplars. However, there were a number of victims that had comparison exemplars that were insufficient or non-existent. Thus, while some of the remains produced a DNA profile that met the minimum threshold requirement, a positive identification was not possible due to the lack of DNA exemplars. Despite these limitations, the WTC-Human Remains Database provided adequate sample sizes to address variation in successful DNA typing rates by sex, victim type, recovery location, and skeletal element. The results demonstrate significant variation in DNA identification rates between skeletal elements, with higher overall successful typing rates among weight-bearing lower limb elements than among elements of the trunk, the upper limb, and head.

Establishing general sampling guidelines that can be tailored for specific contexts will save time, money, and effort, and will ultimately aid in streamlining the identification process. From a mass fatality management perspective, the results of this study suggest that patellae, metatarsals, and foot phalanges are likely to produce successful DNA profiles at rates comparable to femora, tibiae, and ribs in many mass disaster contexts. Given the relative ease of sampling the patellae and foot elements, it may be preferable to select these elements for DNA sampling in future mass fatality incidents.

Acknowledgments

The authors would like to thank the New York City Office of Chief Medical Examiner employees who provided data for this study and the anonymous reviewers for *JFS*.

References

1. Mundorff AZ. Anthropologist-directed triage: three distinct mass fatality events involving fragmentation of human remains. In: Adams B, Byrd J, editors. Recovery, analysis and identification of commingled human remains. Totowa: Humana Press, 2008;123–44.
2. Mackinnon G, Mundorff AZ. The World Trade Center: September 11th, 2001. In: Thompson T, Black S, editors. Forensic human identification: an introduction. Boca Raton: CRC Press, 2006;485–99.
3. Mundorff AZ, Shaler RC, Bieschke E, Mar-Cash EJ. Marrying anthropology and DNA: essential for solving complex commingling problems in cases of extreme fragmentation. In: Adams B, Byrd J, editors. Recovery, analysis and identification of commingled human remains. Totowa: Humana Press, 2008;285–300.
4. Clayton TM, Whitaker JP, Maguire CN. Identification of bodies from the scene of a mass disaster using DNA amplification of short tandem repeat (STR) loci. Forensic Sci Int 1995;76(1):7–15.
5. Ludes B, Tracqui A, Pfitzinger H, Kintz P, Levy F, Disteldorf M, et al. Medico-legal investigations of the Airbus, A320 crash upon Mount Ste-Odile, France. J Forensic Sci 1994;39(5):1147–52.
6. Leclair B, Fregeau CJ, Bowen KL, Borys SB, Elliott J, Fourney RM. Enhanced kinship analysis and STR-based DNA typing for human identification in mass disasters. In: Sensabaugh GF, Lincoln PJ, Olaisen B, editors. Progress in forensic genetics 8. Proceedings of the 18th International ISFH Congress. San Francisco, CA: Elsevier Science, 1999;91–3.
7. Olaisen B, Stenersen M, Mevag B. Identification by DNA analysis of the victims of the August 1996 Spitsbergen civil aircraft disaster. Nat Genet 1997;15(4):402–5.
8. Ballantyne J. Mass disaster genetics. Nat Genet 1997;15(4):329–31.
9. Hsu CM, Huang NE, Tsai LC, Kao LG, Chao CH, Linacre A, et al. Identification of victims of the 1998 Taoyuan Airbus crash accident using DNA analysis. Int J Legal Med 1999;113(1):43–6.
10. Corach D, Sala A, Penacino G, Sotelo A. Mass disasters: rapid molecular screening of human remains by means of short tandem repeats typing. Electrophoresis 1995;16(9):1617–23.
11. Goodwin W, Linacre A, Vanezis P. The use of mitochondrial DNA and short tandem repeat typing in the identification of air crash victims. Electrophoresis 1999;20(8):1707–11.
12. Fernando R, Vanezis P. Medicolegal aspects of the Thai Airbus crash near Kathmandu, Nepal: findings of the investigating pathologists. Am J Forensic Med Pathol 1998;19(2):169–73.
13. Budimlija ZM, Prinz MK, Zelson-Mundorff A, Wiersema J, Bartelink E, Mackinnon G, et al. World Trade Center human identification project: experiences with individual body identification cases. Croat Med J 2003;44(3):259–63.
14. Sledzik PS, Rodriguez WC. Damnum fatale: the taphonomic fate of human remains in mass disasters. In: Haglund WD, Sorg MH, editors. Advances in forensic taphonomy: method, theory, and archaeological perspectives. Boca Raton: CRC Press, 2002;321–30.
15. Graw M, Weisser HJ, Lutz S. DNA typing of human remains found in damp environments. Forensic Sci Int 2000;113(1-3):91–5.
16. Hochmeister MN, Budowle B, Borer UV, Eggmann U, Comey CT, Dirnhofer R. Typing of deoxyribonucleic-acid (DNA) extracted from compact-bone from human remains. J Forensic Sci 1991;36(6):1649–61.
17. Imaizumi K, Miyasaka S, Yoshino M. Quantitative analysis of amplifiable DNA in tissue exposed to various environments using competitive PRC assays. Sci Justice 2004;44(4):199–208.
18. Ye J, Ji AQ, Parra EJ, Zheng XF, Jiang CT, Zhao XC, et al. A simple and efficient method for extracting DNA from old and burned bone. J Forensic Sci 2004;49(4):754–9.
19. Galloway A, Willy P, Snyder L. Human bone mineral densities and survival of bone elements: a contemporary sample. In: Haglund WD, Sorg MH, editors. Forensic taphonomy: the postmortem fate of human remains. Boca Raton: CRC Press, 1997;295–317.
20. Perry WL, Bass WM, Riggsby WS, Sirotkin K. The autodegradation of deoxyribonucleic-acid (DNA) in human rib bone and its relationship to the time interval since death. J Forensic Sci 1988;33(1):144–53.
21. Prinz M, Carracedo A, Mayr WR, Morling N, Parsons TJ, Sajantila A, et al. DNA Commission of the International Society for Forensic Genetics (ISFG): recommendations regarding the role of forensic genetics for disaster victim identification (DVI). Forensic Sci Int: Genetics 2007; 1:3–12.
22. Alonso A, Andelinovic S, Martin P, Sutlovic D, Erceg I, Huffine E, et al. DNA typing from skeletal remains: evaluation of multiplex and megaplex STR systems on DNA isolated from bone and teeth samples. Croat Med J 2001;42(3):260–6.
23. Edson SM, Ross JP, Coble MD, Parsons TJ, Barritt SM. Naming the dead: confronting the realities of rapid identification of degraded skeletal remains. Forensic Sci Rev 2004;16(1):63–90.
24. Milós A, Selmanovic A, Smajlovic L, Huel R, Katzmarzyk C, Rizvic A, et al. Success rates of nuclear short tandem repeat typing from different skeletal elements. Croat Med J 2007;48:486–93.
25. Parsons TJ, Weedn VW. Preservation and recovery of DNA in postmortem specimens and trace samples. In: Haglund WD, Sorg MS, editors. Forensic taphonomy: the postmortem fate of human remains. Boca Raton, FL: CRC Press, 1997;109–38.
26. Lyman RL. Vertebrate taphonomy. Cambridge manuals in archaeology. Cambridge: Cambridge University Press, 1994.
27. Leney MD. Sampling skeletal remains for ancient DNA (aDNA): a measure of success. Historical Archaeology 2006;40(3):31–49.
28. Butler JM. Forensic DNA typing: biology, technology, and genetics of STR markers. 2nd ed, New York: Elsevier Academic Press, 2005.
29. Briggs C, Buck A. The role of the anthropologist in disaster victim identification: the Bali incidents 2002 and 2004. In: Blau S, Ubelaker DH, editors. Handbook of forensic anthropology and archaeology. World Archaeological Congress Research Handbooks in Archaeology. Walnut Creek: Left Coast Press, 2009;407–15.
30. Cockle D, Andrews D, Thompson D. Tsunami Thailand: disaster victim identification. Identification Canada 2005;28(3):4–15.
31. National Institute of Justice. Mass fatality incidents: a guide for human forensic identification. Technical Working Group for Mass Fatality Forensic Identification (NCJ 199758). Washington, DC: US Department of Justice, National Institute of Justice, 2005;79.
32. Budowle B, Moretti TR, Niezgoda SJ, Brown BL. CODIS and PCR-based short tandem repeat loci: law enforcement tools. Proceedings of the Second European Symposium on Human Identification, 1998;73–88. http://www.promega.com/geneticidproc/eusymp2proc/17.pdf
33. Biesecker LG, Bailey-Wilson JE, Ballantyne J, Baum H, Bieber FR, Brenner C, et al. Epidemiology: DNA identifications after the 9/11 World Trade Center attack. Science 2005;310(5751):1122–3.
34. Promega Corporation. PowerPlex® 16 System Technical Manual, Part# TMD012 (03/08). Madison, WI: Promega Corporation, 2008. http://www.promega.com/tbs/tmd012/tmd012.pdf
35. Sullivan KM, Hopgood R, Gill P. Identification of human remains by amplification and automated sequencing of mitochondrial DNA. Int J Legal Med 1992;105(2):83–6.
36. Cash HD, Hoyle JW, Sutton AJ. Development under extreme conditions: forensic bioinformatics in the wake of the World Trade Center disaster. In: Altman RB, Dunker AK, Hunter L, Jung T, Klein T, editors. Pacific symposium on biocomputing 2003. Singapore: World Scientific Printers, 2003;638–53.
37. Westen AA, Gerretsen RRR, Maat GJR. Femur, rib, and tooth sample collection for DNA analysis in disaster victim identification (DVI). Forensic Sci Med Pathol 2008;4:15–21.

Additional information and reprint requests:
Amy Z. Mundorff, M.A.
Department of Archaeology
Simon Fraser University
8888 University Drive
Burnaby, BC V5A 1S6
Canada
E-mail: amy_mundorff@sfu.ca